

NOAA-NATIONAL OCEAN SERVICE

CENTER FOR COASTAL ENVIRONMENTAL HEALTH AND BIOMOLECULAR RESEARCH

FY99 SIGNIFICANT ACCOMPLISHMENTS

MARINE BIOTOXINS PROGRAM

CHARACTERIZATION OF ALGICIDAL BACTERIA TOXIC TO RED TIDE ALGAE

Research on the interaction of bacteria and red tide algae has provided a new means to understand microbial processes leading to the termination of harmful algal blooms. The 16S rRNA gene for two algicidal bacteria has been sequenced. Preliminary analyses indicate one strain is a member of the flexibacter-cytophaga subgroup of the cytophaga/flexibacter/bacteroides (CFB) phylum within the domain Bacteria, while the other strain is a member of the gamma-proteobacteria. Fluorescently-labeled rRNA probes have been designed for both taxa and are being optimized for in situ hybridization. A high-throughput bioassay for guiding fractionation of extracellular bacterial metabolites based on algicidal activity was developed, and has facilitated the chromatographic separation of an algicidal fraction from bacterial culture filtrate. Defining the role of algicidal bacteria in algal bloom termination provides a basis for new generation management efforts necessary to control harmful algal blooms.

ISOLATION OF A DOMOIC ACID PRODUCING DIATOM FROM LOUISIANA SHELF WATERS

Screening of eighteen clonal *Pseudo-nitzschia* cultures established by colleagues at LUMCON revealed production of DA by two of these clones, both identified as *P. pseudodelicatissima* - a species only twice reported as toxic and never verified by mass spectrometry. Both cultures, isolated from LA shelf waters, were confirmed by tandem mass spectrometry to produce DA. Moreover, the patterns of DA production in culture showed the highest cellular toxin levels during exponential growth and the lowest during stationary phase B essentially the opposite of findings reported previously for most other toxic *Pseudo-nitzschia* spp. This pattern is likely due to toxin being excreted into the growth medium during stationary phase; however, since toxin levels are very low, we are currently attempting to reduce our limit of DA detection in seawater to permit the measurement of toxin in the medium. These observations of DA production by exponentially growing cultures have important implications for the toxicity of rapidly growing field populations dominated by this species.

EVIDENCE OF DIARRHETIC SHELLFISH POISON ALONG THE COAST OF MAINE

An extensive field survey conducted along the coast of Maine for diarrhetic shellfish poison activity in blue mussels yielded positive results with the protein phosphatase 2A activity. This is consistent with the contamination with okadaic acid or related congeners. Phytoplankton populations from these areas containing contaminated mussels were dominated by *Dinophysis norvegica*, a known toxic species. Two additional known toxic species of *Dinophysis* were also found in low numbers: *Dinophysis acuminata* and *D. rotunda*. However, all plankton samples were negative for phosphatase inhibitory activity. Examination of the epiphytic communities from areas with toxic mussels revealed the first occurrence of the toxic dinoflagellate *Prorocentrum lima* reported from the Northeast. Analysis of epiphytic samples rich in *Prorocentrum lima* were phosphatase inhibitory active. Subsequent analysis of these samples using LC-MS/MS showed the production of dinophysis toxin-1 (DTX-1) by wild populations of *P. lima*. Additional analyses are underway to determine which okadaic acid congener is responsible for the activity found in the blue mussels. This study has provided the first evidence of DSP toxins in U.S. coastal waters.

TANDEM MASS SPECTROMETRIC IDENTIFICATION OF DOMOIC ACID: ABSOLUTE IDENTIFICATION OF DOMOIC ACID IN CALIFORNIA SEA LIONS

A newly developed liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was used to provide positive identification of domoic acid in living marine resources and protected species following a toxic bloom of *Pseudo-nitzschia australis* that occurred in spring 1998 in Monterey Bay, CA. The toxic bloom was associated with a sea lion mortality event that resulted in the loss of over 50 animals. The LC-MS/MS method is based upon the

detection of highly specific fragmentation products from the collisionally induced dissociation of domoic acid pseudo-molecular ions. The LC-MS/MS method allowed sensitive detection of domoic acid in a variety of matrices ranging from sea lion tissue and excrement to other components of the food web, including planktivorous fish (e.g., anchovies). This is the first utilization of LC-MS for domoic acid identification and is substantial advance in detection methodology because it provides the absolute identification of toxin that is not possible with functional assays or other HPLC-coupled detection methods.

BIOMONITORING BREVETOXIN EXPOSURE IN MAMMALS USING BLOOD SPOT CARDS

We have developed a method to monitor the exposure of mammals to brevetoxins. This sampling involves collecting whole blood, applying the blood to a ½ inch diameter circle on a specially prepared blood collection card and allowing it to dry. The blood spots are extracted in the laboratory and total brevetoxin activity quantified using high throughput receptor binding assay and specific brevetoxin congeners analyzed by liquid chromatography-tandem mass spectrometry. Toxicokinetic characterization has been conducted with laboratory mice. Mice were treated with 180 ug/kg brevetoxin-3. Whole blood was collected at time points between 0.5 and 24 hours of brevetoxin exposure and 0.1 ml was spotted on filter paper cards. Brevetoxin activity as determined by receptor assay increased between 0.5 and 4.0 hours and was decreased, yet detectable 24 hours after brevetoxin exposure. Tandem mass spectrometry was used to provide confirmation of brevetoxin-3. The mass spectrometry results paralleled those of receptor assay for time points between 0.5 and 4.0 hours exposure. However, brevetoxin-3 was not detected at 24 hours suggesting metabolism to another biologically active form of the toxin. We anticipate that this approach will provide a method to biomonitor for brevetoxins in living marine resources, protected species, and humans and are evaluating this biomonitoring method for other marine toxins as well.

BREVETOXINS INDUCE EMBRYO TOXICITY AND DEVELOPMENTAL ABNORMALITIES

Brevetoxins are lipophilic polyether toxins with documented neurotoxic effects on adult animals. In this study, we extend last years study of ciguatoxin to quantify the adverse developmental effects of brevetoxins using an exposure paradigm that parallels the maternal-oocyte transfer of toxin. Medakafish (*Oryzias latipes*) embryos are exposed to brevetoxin six hours post fertilization by microinjection of a small quantity (2 nanoliters) of brevetoxin (or vehicle) reconstituted in a fish oil (triolein) droplet. The brevetoxin-containing droplet is placed adjacent to the larger oil droplet of the yolk sac. Embryos microinjected with doses of 0.8 ng/egg (ppm) and higher of brevetoxin-1 exhibit pronounced cardiovascular (tachycardia) and muscular (hyperkinesis) activity by embryonic day four. Prior to hatching, morphological abnormalities were commonly found in embryos at the following lowest adverse effect levels: 1.1 ppm- lateral curvature of the spinal column; 3.1 ppm- herniation of brain and meninges though defects in the skull; and 3.4 ppm malpositioned eye. Hatching abnormalities are also commonly observed at brevetoxin doses of 2.0 ppm and higher with head-first, as opposed to the normal tail-first hatching. The observation of developmental abnormalities following brevetoxin exposure identifies a new spectrum of adverse effects that may be expected to occur following exposure to red tide events.